# Design of a multicellular feedback control strategy in a synthetic bacterial consortium

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***Abstract*— Living organisms employ endogenous negative feedback loops to maintain homeostasis despite environmental fluctuations. An intriguing challenge in Synthetic Biology is that of designing and implementing synthetic circuits to control host cells' behavior, thus mimicking what natural evolution has refined and conserved. The high degree of circuit complexity required to accomplish this task, and the intrinsic modularity of classical control schemes, suggest the implementation of synthetic endogenous feedback loops across more than one cell population. The distribution of the sensing, computation and actuation functions required to achieve regulation, to different cell populations within a consortium allows to reduce the genetic engineering in a particular cell and to increase the robustness as well as the possibility of reusing the synthesized circuits. Here we propose and study, *in-silico*, the design of a synthetic microbial consortium implementing a feedback controller across two cell populations.**

## I. Introduction

The majority of living organisms can maintain homeostasis despite external stimuli or environmental fluctuations. The hormone secretion and signaling pathways functioning in multicellular organisms [1], as well as the control of bacterial chemotaxis [2] are only few examples in which the internal state of biological systems is regulated or maintained by employing negative feedback.

Experimental approaches have been proposed to implement exogenous negative feedback control schemes to achieve real time control of gene expression in living cells [3], [4], [5], [6], [7], [8]. In all these cases, the experimental setup consisted of a device to grow cells, a sensing apparatus (cytofluorimeter or fluorescence microscope) to monitor cells' behavior (quantify fluorescence from fluorescent reporters), a PC running control algorithms and a set of actuators to provide inputs to the cells according to the control objective and their current status.

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An open challenge in Synthetic Biology is that of synthesizing endogenous negative feedback controllers, where all the sensing, computation and actuation functions are embedded in living cells. The range of possible applications of synthetic regulators can span from the optimization of chemical production in bioreactors [9], [10], to targeted drug delivery in multicellular organisms [11].

The implementation of synthetic feedback controllers in single cells has been proposed with the aim of performing set-point regulation [12], [13], or signal tracking control [14] of target proteins. These complex functions are achieved through the 'wiring'of numerous parts that are generally difficult to characterize and integrate all together in a single cell, with also a resulting metabolic burden that can be self-defeating for the host [15]. Moreover, this approach to the implementation of biological regulators leads to the design of parts that cannot be easily adapted and reused for different control applications [16].

In order to overcome these drawbacks, inspired by the intrinsic modularity of classical control schemes, we propose to distribute the sensing, computation and actuation functions to different cell populations within a consortium. Recently, the construction and the study of a synthetic oscillator implemented across two distinct cell types has clearly shown how the genetic engineering of interacting microbial populations can be exploited to achieve complex and robust population-level behaviors [17]. Indeed, the interaction of microbial populations can be advantageous in accomplishing complicated tasks better than a single colony can do, and also beneficial to guarantee robustness to environmental fluctuations [18], [19].

Here we present and study the *in-silico* implementation of a multicellular feedback controller in a consortium of two interacting populations. We provide an *in-silico* proof-of-concept of the whole design and discuss the experimental setup needed for its *in-vivo* implementation.

## II. Multicellular feedback control design

The proposed implementation consists of two interacting microbial populations, where specifically one population (the 'Controllers') embeds synthetic circuits to sense and control the status of a process in the other ('the Targets'). The Controllers can receive an external signal (e.g. an inducer molecule) so that the desired reference level of the process to be regulated in the Target cells can be set (Fig. 1). The two populations communicate with each other through the control

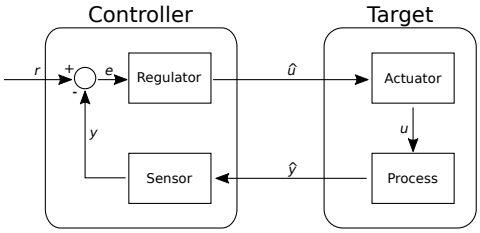


Fig. 1. **Proposed feedback control strategy.** Controller cells embed the sensor to get information on the process status in the Targets and a regulator to provide inputs to the Targets according to the deviations between the reference $r$ and the measured output $y$.

input $\hat{u}$ (from the Controllers to the Targets), and the process output $\hat{y}$ (fed back from the Targets to the Controllers).

An abstract biological implementation of the feedback control strategy is shown in Fig. 2. Communication within the synthetic consortium is achieved via the release of signaling molecules into the growth medium. An external reference signal inhibits the production of the species $A$ in the Controllers whose complex with the signaling molecule $Q_2$ generates $B$ which catalyzes the synthesis of another signaling molecule, $Q_1$, that is released in the growth medium as the input signal. $Q_1$ can diffuse across the membrane of the Targets and activate the production of $C$ which in turn inhibits the output spieces $D$ whose concentration has to be regulated towards the level set by the reference. The feedback loop is thus closed by $D$ which catalyzes the synthesis of the molecule $Q_2$ whose concentration in the growth medium is interpreted by the Controllers as the system output readout. The proposed topology is such that the input signal ($Q_1$ concentration) is an indirect function of the deviation between the reference signal and the output readout ($Q_2$ concentration). In what follows we denote by $Q_{1,e}$, $Q_{1,c}$ and $Q_{1,t}$ the concentration of the input signal outside the cells, in the Controller cells and in the Target cells, respectively, and adopt a similar notation for $Q_{2,e}$, $Q_{2,c}$ and $Q_{2,t}$.

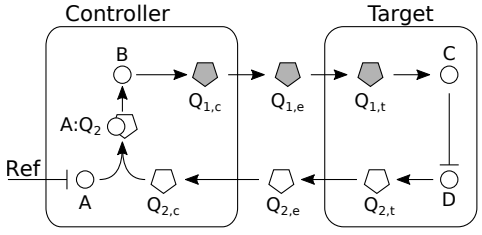


Fig. 2. **Multicellular feedback control design.** Abstract biological implementation of the multicellular feedback consortium.

## III. Model derivation and parameterization

### A. Model derivation

The concentrations of species $B$, $C$, $D$, and of the active complex $A{:}Q_2$ in the gene regulatory networks (GRNs) in the Controller and in the Target cells are modeled by ODEs, while spatial diffusion of the signals between populations is modeled by means of Partial Differential Equations (PDEs).

Activation, or repression, of each species $x$ by its regulator $s$ is governed by a Hill function with dissociation constant $K_s$ and exponent $n_s$. The active complex $A{:}Q_2$ is simultaneously activated by the signaling molecule $Q_2$ and repressed by the incoming reference signal $r(t)$ (i.e. is regulated by two independent and competing species), and is concisely modeled as the product of two Hill functions [20]. Degradation of species $x$ is governed by first order kinetics with corresponding rate $\gamma_x$. Moreover, for each species $x$, transcription and translation characteristic parameters are embedded in the basal ($\chi_{x,0}$) and maximal ($\chi_x$) activity constants.

*a) Controller population:* The regulated product $B$ is only produced in response to the active complex $A{:}Q_2$, and is not induced by the individual inactive constituent components of this complex. The dynamics of the species' concentrations in the Controller cells can be written as:

$$\begin{aligned}\frac{d[A{:}Q_2]}{dt} &= \left(\chi_{A:Q,r,0} + \chi_{A:Q,r}\frac{{K_r}^{n_r}}{{K_r}^{n_r}+[\mathrm{Ref}]^{n_r}}\right)\cdot \\ &\cdot\left(\chi_{A:Q,a,0} + \chi_{A:Q,a}\frac{[Q_{2,c}]^{n_q}}{{K_q}^{n_q}+[Q_{2,c}]^{n_q}}\right) - \gamma_{A:Q}[A{:}Q_2],\end{aligned} \tag{1}$$

$$\frac{d[B]}{dt} = \chi_{b,0} + \chi_b\frac{[A{:}Q_2]^{n_b}}{{K_b}^{n_b}+[A{:}Q_2]^{n_b}} - \gamma_B[B]. \tag{2}$$

*b) Target population:* Targets receive the control input from the Controllers in the form of $Q_1$ concentration. This catalyzes the synthesis of species $C$, which in turn inhibits $D$. Specifically the dynamics of the species' concentrations in the Target cells can be described by:

$$\frac{d[C]}{dt} = \chi_{c,0} + \chi_c\frac{[Q_{1,t}]^{n_c}}{{K_c}^{n_c}+[Q_{1,t}]^{n_c}} - \gamma_C[C], \tag{3}$$

$$\frac{d[D]}{dt} = \chi_{d,0} + \chi_d\frac{{K_d}^{n_d}}{{K_d}^{n_d}+[C]^{n_d}} - \gamma_D[D]. \tag{4}$$

*c) Communication:* The two populations communicate via two pathways, the former directed from the Controllers towards the Targets (pathway 1, molecule $Q_1$), and the latter going from the Targets to the Controllers (pathway 2, molecule $Q_2$). The two pathways are symmetrical, indeed for each of them a sender and a receiver cell population can be identified. Furthermore, the following assumptions are made in order to model the dynamics of $Q_1$ and $Q_2$:

1) no cross-talk is present between different signals;
2) the two molecules diffuse across the cell membranes with the same diffusion coefficient $\eta$ and are degraded in the cells at the same rate $\gamma_i$;
3) the intra-cellular concentration in the sender depends on the rate of production of the molecule ($K_Q$), on the exchange with the extra-cellular environment and on the degradation inside the cell;
4) the intra-cellular concentration in the receiver is a function of the exchange with the extra-cellular environment and of the degradation inside the cell;
5) the extra-cellular concentrations are function of the diffusion coefficient $\Theta$ in the growth medium, of

the exchange between the cells and the extra-cellular environment and of the external degradation rate $\gamma_e$.

The dynamics of the intra-cellular and external concentrations of $Q_1$ can therefore be described as:

$$\frac{d[Q_{1,c}]}{dt} = K_{Q_1}[B] + \eta([Q_{1,e}] - [Q_{1,c}]) - \gamma_i[Q_{1,c}], \quad (5)$$

$$\frac{d[Q_{1,t}]}{dt} = \eta([Q_{1,e}] - [Q_{1,t}]) - \gamma_i[Q_{1,t}], \quad (6)$$

$$\frac{\partial[Q_{1,e}]}{\partial t} = \eta([Q_{1,c}] - [Q_{1,e}]) + \eta([Q_{1,t}] - [Q_{1,e}]) + -\gamma_e[Q_{1,e}] + \Theta\nabla^2[Q_{1,e}]; \quad (7)$$

while those for $Q_2$ as:

$$\frac{d[Q_{2,c}]}{dt} = \eta([Q_{2,e}] - [Q_{2,c}]) - \gamma_i[Q_{2,c}], \quad (8)$$

$$\frac{d[Q_{2,t}]}{dt} = K_{Q_2}[D] + \eta([Q_{2,e}] - [Q_{2,t}]) - \gamma_i[Q_{2,t}], \quad (9)$$

$$\frac{\partial[Q_{2,e}]}{\partial t} = \eta([Q_{2,c}] - [Q_{2,e}]) + \eta([Q_{2,t}] - [Q_{2,e}]) + -\gamma_e[Q_{2,e}] + \Theta\nabla^2[Q_{2,e}]. \quad (10)$$

### B. Parameterization

Model parameterization is carried out according to characteristic ranges of values available in the literature to describe the dynamics of the interacting species of the proposed GRNs [21], [22], [23]. The specific values selected are indicated in Table I.

TABLE I
PARAMETER VALUES FOR ALL NUMERICAL SIMULATIONS.

| Parameter | Value | Description |
|---|---|---|
| $\chi_{0,x}$ | 1e-1 $\mu$M min$^{-1}$ | Baseline production of species $x$ |
| $\chi_x$ | 2 $\mu$M min$^{-1}$ | Maximal production of species $x$ |
| $\gamma_x$ | 1.4 min$^{-1}$ | Degradation of species $x$ |
| $n_a$ | 2 | Hill coeff., for $a \in r,q,b,c,d$ |
| | | Dissociation constants in equations: |
| $K_r$ | 1 $\mu$M | (1) |
| $K_q$ | 0.1 $\mu$M | (1) |
| $K_b$ | 0.5 $\mu$M | (2) |
| $K_c$ | 0.015 $\mu$M | (3) |
| $K_d$ | 0.5 $\mu$M | (4) |
| $K_Q$ | 0.05 min$^{-1}$ | Synthesis rate of $Q_{1,2}$ |
| $\eta$ | 2 min$^{-1}$ | Cell wall diffusion rate of $Q_{1,2}$ |
| $\gamma_i$ | 0.4 min$^{-1}$ | Internal $Q_{1,2}$ degradation |
| $\gamma_e$ | 0.2 min$^{-1}$ | External $Q_{1,2}$ degradation |
| $\Theta$ | 800 $\mu$m$^2$ sec$^{-1}$ | external diffusion rate of $Q_{1,2}$ |

## IV. IN-SILICO EXPERIMENTS

The feedback control strategy is simulated for two different configurations of the interacting modules: *a) aggregate population scenario*, where each of the two populations (Controllers and Targets) is modeled as a single average cell, and *b) agent-based scenario*, in which individual cells' dynamics are simulated via an agent-based interaction model.

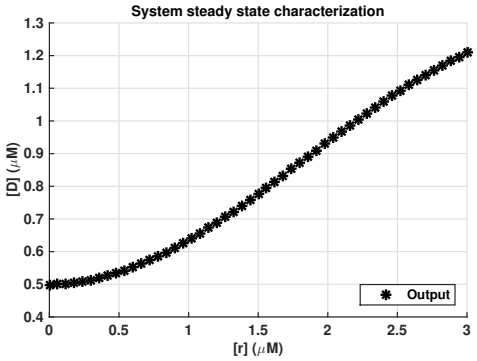


Fig. 3. **Aggregate scenario, steady-state characterization.** System steady-state values calculated when the control reference is varied quasi-statically in the range $[0,3]\mu M$ (black dots), and first order polynomial fitting of the output data (grey solid line).

### A. Aggregate scenario

The aim of the analysis in this configuration is to test the overall behavior of the system in response to different control references (set-point regulation and signal tracking control) in the presence of spatial diffusion of the inducer molecules between the Controller and Target cells, to assess the robustness of the proposed control strategy and to quantify the effect of controller parameter variations.

In this scenario a single Controller is assumed to interact with a single Target in a mono-dimensional spatial domain where the two cells are located at a distance of $20\mu m$ (compatible with experimental conditions in standard microfluidic devices [24]). Equations (7) and (10) are discretized over the $N$ points in which the spatial domain is divided by means of the finite differences using the central step discretization method. Dirichlet's boundary conditions are imposed in order to solve the resulting system of $2N$ ODEs. The equations resulting from the discretization of (7) and (10) in the space domain and the other ODEs describing the full system are integrated with the ode15s MATLAB solver.

*a) System steady state characterization:* The relationship between the control reference signal and the process output ($D$ concentration) at steady-state is nonlinear as shown in Fig. 3, where the reference signal is varied quasi-statically between $0\,\mu M$ and $3\,\mu M$.

The resulting response is significantly nonlinear for concentrations of the control reference signal in the interval $[0,1.5]\,\mu M$. This nonlinear characterization is then used to set the reference signal in order to calculate the desired behavior in control experiments.

*b) Control performance:* The following reference signals are considered in order to assess the performance of the regulation strategy proposed:

- *multi set-point signal:* $r(t) = u(t-300) + 2\cdot u(t-700) - u(t-1100)$;
- *trapezoidal signal:* $r(t) = \alpha\cdot(t-100)\cdot u(t-100) - \alpha\cdot(t-500)\cdot u(t-500) - \alpha\cdot(t-900)\cdot u(t-900) + \alpha\cdot(t-1300)\cdot u(t-1300)$;
- *sinusoidal signal:* $r(t) = u(t-300) + \sin\left(\frac{2\cdot\pi\cdot(t-300)}{400}\right)$;

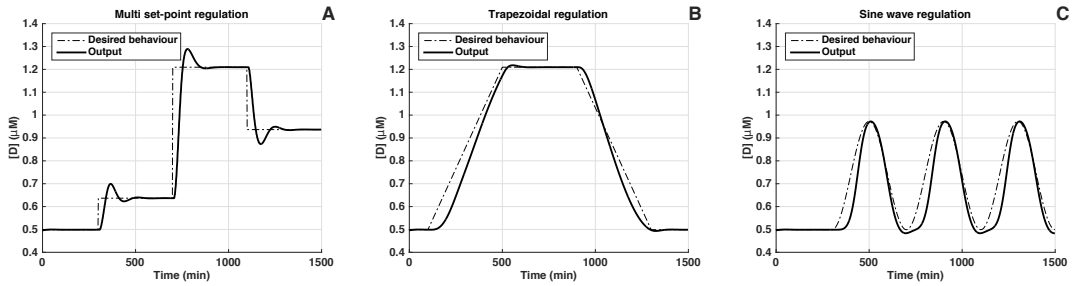


Fig. 4. **Aggregate scenario, control results.** System dynamic response for the multi-step (A) trapezoidal (B) and sinusoidal (C) control references. Concentration of the output species $D$ (solid line) and the desired output (dashed line) are plotted for each of the control reference signals.

where $u(t)$ is the Heaviside step function and, $\alpha$ is the slope of the linear increasing and decreasing sections of the trapezoidal signal.

Despite the presence of strong nonlinearities in the model and the explicit modeling of spatial diffusion of the signaling molecules, the multicellular feedback control strategy is effective in achieving set-point regulation with extremely low steady-state error, an overshoot less than the 10% of each desired output value and a settling time of about 150 minutes (Fig. 4 A).

Also, tracking of both the trapezoidal and the sinusoidal control reference signals is acceptable with good matching between the achieved output and the reference wave-forms (Fig. 4 B and C).

*c) Robustness analysis:* Next, robustness of the proposed multicellular feedback control strategy is assessed by considering perturbations of the model parameters in a set-point regulation scenario.

In particular simulations are carried out by choosing the parameters of the Target cells' GRN and of the signaling molecules' diffusion dynamics [equations (3),(4), (6),(7) and (9),(10)] from normal distributions centered in the corresponding nominal values and with standard deviations equal to 5%, 10% and 20%.

Despite the perturbations, as shown in (Fig. 5), the control performance continues to be acceptable with the worst case being observed, as expected, when the largest parameter perturbation is considered.

*d) Controller tuning:* The effects of parameter variation in the proposed model are further investigated in order to assess whether and how the control performance is affected by changes in the parameters of the Controller cell population.

The modulation of the ratios $\delta_B = \frac{\chi_B}{K_b}$ and $\delta_{Q_1} = \frac{K_{Q_1}}{\gamma_i}$ with respect to their nominal values is tested, by varying $K_b$ and $\gamma_i$ in order to double or halve the corresponding ratios. Results depicted in Fig. 6 suggest that an increase of the dissociation constant associated to species $B$ as well as of the internal degradation of $Q_1$ can reduce the overshoot and the settling time. However in the latter case, as expected since $Q_1$ is degraded faster, the output dynamic range is significantly decreased (20% less of the nominal output). Moreover decreasing $K_b$ and $\gamma_i$ leads to an increase of the settling time and to a reduction of the output dynamical range (40% and 15% respectively).

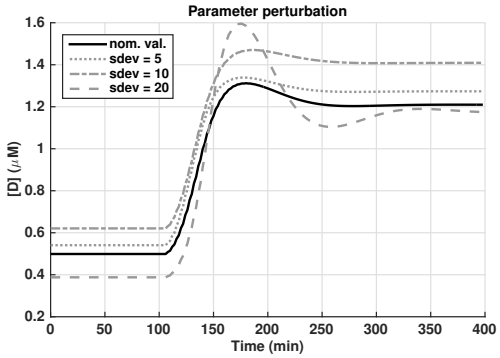


Fig. 5. **Aggregate scenario, robustness analysis.** Results for the set-point regulation achieved when parameters in equations (3)-(4) and (6)-(10) are perturbed as described in the text.

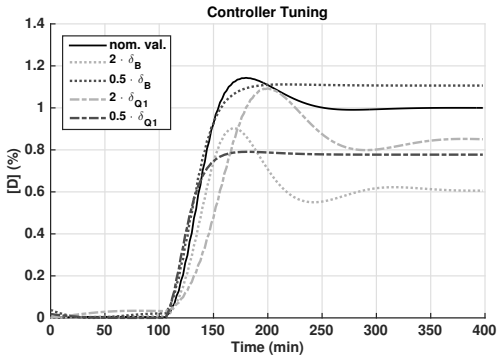


Fig. 6. **Aggregate scenario, perturbation of the Controllers' parameters.** Results for the set-point regulation achieved when the ratios $\delta_B = \frac{\chi_B}{K_b}$ and $\delta_{Q_1} = \frac{K_{Q_1}}{\gamma_i}$ are doubled or halved.

## *B. Agent-based scenario*

The control performance is tested next in a realistic multicellular environment with an agent-based interaction model using the BSim framework [25]. Cells are represented using BSim's *E. coli* bacterium model, with biologically realistic cell motility [26]. Extra-cellular environment is modeled as a microfluidic chamber with dimensions of 40x50x1 $\mu m^3$ able to host a maximum of 480 cells [24], open to the external flow only on one short side allowing for diffusion of a reference signal into the chamber and diffusion of the signaling molecules out of the chamber, on that side only

[23].

Two cell types are defined for the Controller and Target agents, and the relevant equations for the corresponding GRNs are individually embedded in each cell type, and solved using a fourth order Runge-Kutta solver. Parameters for the GRNs are kept at the same values used in the aggregate scenario. The external concentrations of signaling molecules are implemented by finite volume discretization of the corresponding PDEs over a grid across the simulation domain. Cell growth and division are not taken into account.

Moreover, the effect of reducing cell density from the maximal total number of cells in the simulation volume is first investigated. Secondly, while maintaining the total cell density at the maximal value, the impact on the control outcome of reducing the fraction of Controller cells is analyzed.

*a) Signal tracking performance:* The agent-based model is simulated using the two time-varying reference signals defined in the previous section (Fig. 4 B and C).

A control performance comparable to that seen in the aggregate scenario can be observed in Fig. 7. The output averaged across the Target population tracks the reference signal closely, for both input wave forms, with a negligible delay. Moreover, the standard deviation of the response across the population is less than 1% of the mean (data not shown).

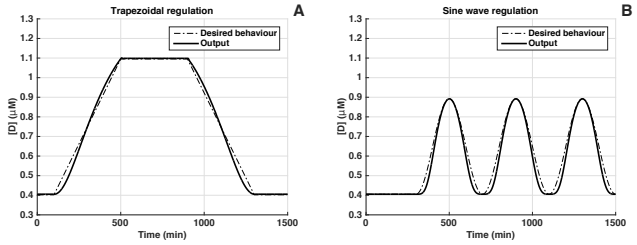


Fig. 7. **Agent-based scenario, signal tracking control.** Agent-based control results in response to a trapezoidal reference signal (A) and sinusoidal control reference (B).

*b) Population effects on multi-level set point control:* The agent-based system is simulated with the same multi-level set point reference input as shown in Fig. 4 A.

The total population density is first perturbed (Fig. 8), and then the effect of varying the proportion of Controller cells in the total population is assessed while keeping the total population at the maximum level of 480 cells (Fig. 9). Cell density and Controllers' ratio both have a significant impact on the regulation outcome, since the amount of the available control input (molecule $Q_1$), as well as its effect are reduced when cells are less packed or when fewer Controllers are present in the microfluidic chamber. The decreasing of cell density and of the Controllers/Targets ratio is associated to a reduction of the overshoot and of the oscillations observed in the system's response but also, for values less than 0.25 and 0.1 respectively, to an unwanted drop of the output dynamic range. The best control outcome, corresponding to reduced overshoot and oscillations as well as to the expected output dynamic range, is achieved when the Controllers/Targets ratio is set to 0.1 and the population density is nominal

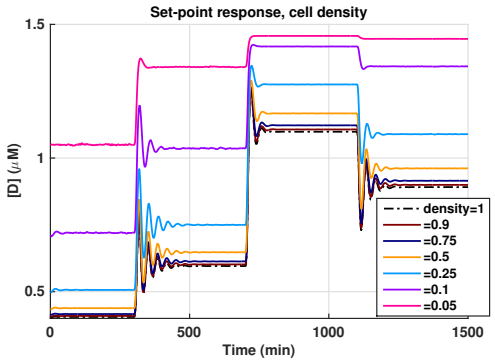


Fig. 8. **Agent-based scenario, effects of variable cell density.** Average system output achieved for multi-level set-point control task for different population densities.

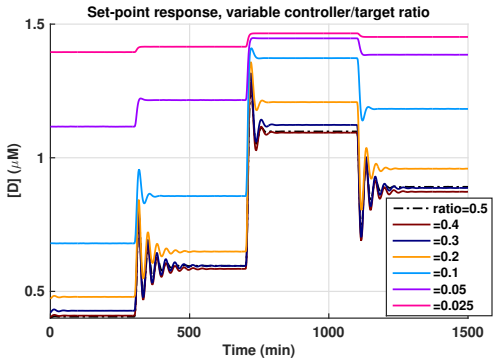


Fig. 9. **Agent-based scenario, effects of variable Controllers/Targets ratio.** Average system output achieved for multi-level set-point control task for different Controller/Target ratios.

## V. Discussion

We have described the *in-silico* implementation of a multi-cellular feedback control strategy within a synthetic cellular consortium where a population acts as a controller trying to regulate the concentration of a specific molecular species in the other. The two cell populations can communicate via the release of signaling molecules in the shared growth medium and a control reference signal can be provided to the controller population by means of an external inducer molecule.

We have proposed a mathematical representation of the two interacting cell populations considering characteristic parameters of standard biological parts employed in Synthetic Biology. The *in-silico* experiments have shown the effectiveness of the implemented feedback controller, despite the parts composing the whole circuit being spatially separated and thus the signals (control input and system output) attenuated by diffusion and propagation in the extracellular environment. We have investigated the robustness of the proposed approach to parameter perturbations and analyzed the scaling effects of increasing the two population

sizes and of varying their ratio in a realistic simulation environment. The robustness analysis has shown that the presented multicellular feedback control strategy is robust to large parameter variations. This suggests that the same controller population could be used to regulate different synthetic target cells. Also, *in-silico* experiments provided guidelines on how to tune the Controllers' parameters in order to improve control performance.

In the ongoing biological implementation of the proposed control strategy in a consortium of *E. coli* cells, custom designed microfluidic devices are employed not only to grow the cells, but also to regulate/set the relative ratio of the two populations as this has been shown to be particularly relevant in our *in-silico* predictions to optimize the final outcome. Orthogonal quorum sensing systems are being exploited to implement communication across the two populations within the consortium [27]. Moreover, in a realistic scenario the interaction between the inducer molecule acting as the external control reference and the parts within the controller population could be mediated by an intermediate species which in turn affects the production of the regulator forming the complex with the signaling molecule coming from the target cells [28].

On the basis of our predictions and of the engineering of microbial communities completed so far [17], [18], [27], [29], we believe that the implementation of a feedback control scheme in a synthetic consortium of living bacteria could provide a useful tool for the realization of robust and versatile embedded cellular controllers. Further *in-silico* studies tackling the stochasticity of biochemical processes and experimental work towards the implementation of the proposed strategy are currently being carried out and will be reported elsewhere [30].

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